

<http://www.pjbs.org>

**PJBS**

ISSN 1028-8880

**Pakistan  
Journal of Biological Sciences**

**ANSI***net*

Asian Network for Scientific Information  
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

## The Study of Adrenal Chromaffin of Fish, *Carassius auratus* (Teleostei)

M. Sampour

Department of Biology, University of Luristan, Khoram Abad, Iran

---

**Abstract:** In *C. auratus* the adrenal chromaffin tissue is situated around the posterior cardinal veins, in the head kidney. Chromaffin tissue consists of two types of cells containing secretory granules, adrenaline and noradrenaline cells. The cells produced catecholamine hormones. Adrenaline cell contains electron-lucent granules, whereas noradrenaline cells possess electron-dense granules. Cholinergic fibers embedded in the head kidney innervate the chromaffin cell. Two types of secretory structures, synaptic vesicles and secretory granules are found within the presynaptic terminal. Secretory granules discharge their contents, as neuropeptide in non-synaptic area of nerve terminal by exocytosis, whereas synaptic vesicles discharge their contents as neurotransmitters at the synaptic thickening (active zone) in the presynaptic terminal by exocytosis.

**Key words:** *Carassius auratus*, chromaffin cells, synaptic vesicle, secretory granule, adrenaline cell, noradrenaline cell

---

### INTRODUCTION

There has so far been considerable attention in studying on adrenal gland of fish. Most studies have focused on the adrenal interrenal tissue. Little attention has been pointed to investigation of functional ultrastructure of the adrenal chromaffin of fish. In fish interrenal and chromaffin tissues are separated from each other and are scattered in kidney region, around the blood vessels. The position tissues varies in different groups of fishes. In lamprey (cyclostomata) the chromaffin cells occur along side of the lateral walls of both anterior and posterior cardinal veins near their union (Epple and Potter, 1985) and in dogfish chromaffin tissue forms two rows located along the inner border of the kidneys and the interrenal tissues are placed between them. Withes (1992) reported that in elasmobranchs, chromaffin and interrenal components are entirely separated from each other and the chromaffin cells cluster along side the inner border of kidney and the more posterior ones are embedded in the kidney. In teleosts as in other fishes adrenal glands are represented by two distinct tissues interrenal and chromaffin, which are situated in the kidney region, but remain separated from each other. Two components are homologous with the adrenal cortex and medulla respectively in mammals. Most of chromaffin cells are embedded in the wall of large veins. Also chromaffin cells may be intermingle with the interrenal tissue. The morphology and distribution of the chromaffin cells have been examined in many species in fishes (Young, 1993; Scheuermann, 1993). The histochemical of adrenal gland of several species of teleostei has been investigated by some researchers. Gallo *et al.* (1993) investigated the cytology and biochemical of chromaffin cells in some

teleostei. Perry and Bernier (1999) reported that in teleost fishes, adrenalin and noradrenaline hormones are synthesized and released by chromaffin cells lining posterior cardinal vein. The neurotransmitters Pituitary Adenyls Cyclase-Activating Polypeptide and Vasoactive Intestinal Polypeptide are potent secretagogues of adrenal catecholamine secretion in mammals (Lamouche and Yamaguchi, 2001) and in trout fish (Montpetit and Perry, 2000). They reported that in trout the mentioned neuropeptide are released from the preganglionic fibres innervating the chromaffin cells under low-frequency nerve stimulation conditions. Some investigations have been carried out on nerve terminal of chromaffin cells of teleostei fishes by Imagawa *et al.* (1996) holostean by Youson (1976) and dipnoan by Scheuermann (1993). In *Brycon cephalus*, chromaffin cells are found in small groups, closely associated with the interrenal gland (Rocha *et al.*, 2001). In *C. auratus*, the chromaffin cells occur around the posterior cardinal in head kidney and are vascularized by small branches of the dorsal. No ultrastructural studies have been carried out on *C. auratus*, previously. In the present investigation the fine structure of adrenal chromaffin of *C. auratus* is reported.

### MATERIALS AND METHODS

Specimens of *C. auratus* were killed by decapitation, the most of visceral, except for head kidney and blood vessels (including the posterior cardinal vein) were removed from the body cavity. The body cavity was infused with saline solution including 1% tannic acid, the specimens were fixed with 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate (pH 7.8) for 1 h. Then

the head kidney was removed from the body cavity, then was post-fixed in 1% Osmium tetroxide ( $\text{OsO}_4$ ) in 0.1 phosphate buffer (pH 7.4) for 1 h, washed in a solution containing sodium cacodylate and distilled water for 1 h, dehydration was carried out in a series of different percentage of acetone solutions for 15 min. Then the tissue were embedded in a fresh Epon resin. Thick section were sliced at  $1 \mu\text{m}$  and thin sections sliced at  $60 \text{ nm}$  thickness. Ultrathin sections were mounted on uncoated copper grids. Sections were stained in aqueous uranylacetate and lead citrate. Finally the thin sections were viewed in a Joel transmission electron microscopy operated at 60 KV. This study was carried out in 2004, in Khoramabad, Iran.

## RESULTS

The adrenal gland of *Carassius auratus* consists of interrenal and chromaffin tissues. Interrenal and chromaffin tissues are located along the posterior cardinal vein. Chromaffin tissue is placed around the posterior cardinal vein in the anterior kidney (head kidney). Some parts of tissue closely associated with interrenal tissue. The fine structural of analysis of the chromaffin tissue reveals that the tissue consists of adrenaline and nor adrenaline cells (Fig. 1). The chromaffin cells are polygonal in shape, they are distinguished by presence of granules, which occupies the whole cytoplasm of cells (Fig. 1). Chromaffin cells are organized into cords and surrounded by nerves. Each cell is surrounded by an obvious plasma membrane, which separates the cell from adjacent cells (Fig. 2). Chromaffin cell contains distinct nucleus, which is relatively large and possesses a prominent nucleolus (Fig. 2). The nucleus varies in shapes (round, oval and multilobed), it occupies a substantial proportion of the cells. In cells nuclear membrane is clearly visible and separated the nucleus from cytoplasm (Fig. 2). The pores are visible on the nuclear membrane. The cytoplasm of both chromaffin cells, contains numerous mitochondria, elongated, oval and rounded in shapes, with tubular cristae, in electron opaque matrix (Fig. 3). In most of mitochondria, the external membrane is clearly visible and cristae extending from inner membrane into the matrix of mitochondria (Fig. 3). Golgi complex consists of vesicles and cisternae, is present in the cytoplasm of chromaffin cells (Fig. 4). The granules at different size and densities are seen in vicinity of Golgi sacculi. The origin of the granules may be Golgi complex with co-operation of reticulum endoplasmic. Some microtubules in small number and parallel to each other are present in the cytoplasm of chromaffin cells. Smooth and rough endoplasmic reticulum are seen in different parts of cells. There are ribosomes situated on the rough endoplasmic reticulum

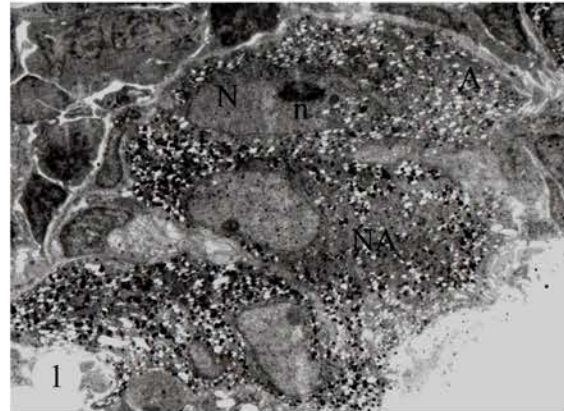


Fig. 1: Exhibiting chromaffin cells, adrenaline (A) and noradrenaline (NA) cells. N, nucleus; n, nucleolus

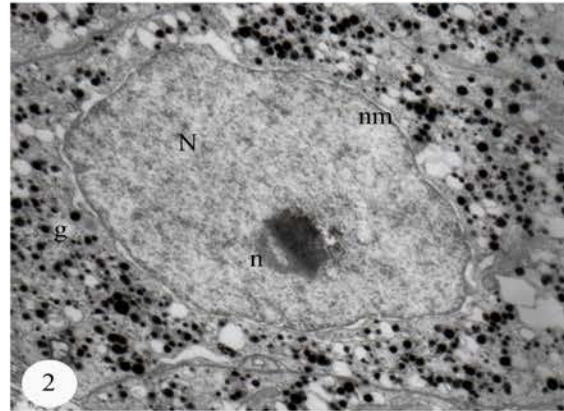


Fig. 2: Exhibiting fine structure of chromaffin cell. N, nucleus; n, nucleolus; nm, nuclear membrane; g, granules

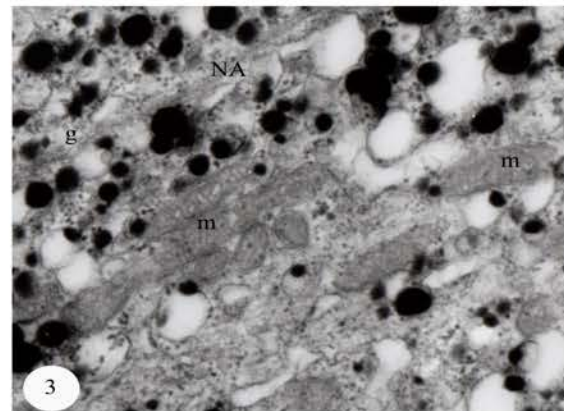


Fig. 3: Indicating nor adrenaline cell (NA). Mitochondria (m) in different shapes are present in the cytoplasm of cell. g, nor adrenaline granules

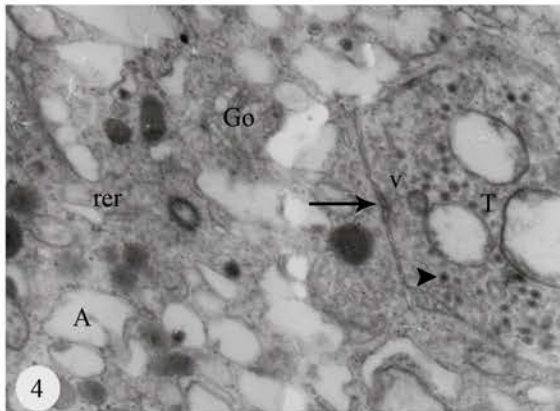


Fig. 4: Fine structure of adrenaline cell (A) and its organelles. Go, Golgi complex; rer, rough endoplasmic reticulum; T, synaptic terminal; v, synaptic vesicles; Synaptic thickening (arrow) and secretory granules (arrow head) are present in the synaptic terminal

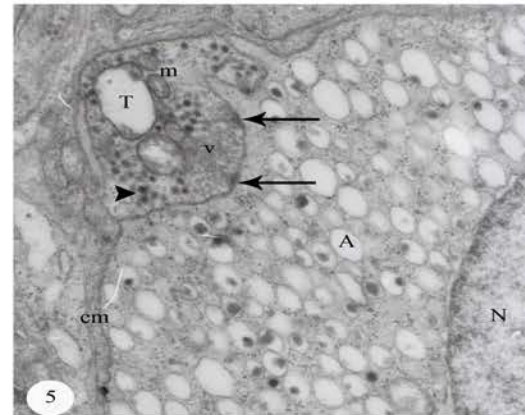


Fig. 5: Indicating synaptic terminal (T) which connected to chromaffin cell (adrenaline, A) and innervated it. N, nucleus of adrenaline cell; v, synaptic vesicles; m, mitochondria. Active zone in the presynaptic (arrows) of nerve ending and secretory granules (arrow head) are present in the synaptic terminal

and free ribosomes in the cytoplasm of cells. It is possible to recognize of adrenaline and nor adrenaline cells from each other with light and electron microscope. The micrographs, which have been, provided by electron microscopy, indicated that nor adrenaline cells are dark whereas, adrenaline cells are light in colour (Fig. 3). Adrenaline cell is characterized by electron-lucent large granules, while nor adrenaline cell is characterized by strongly electron-dense small granules (Fig. 5). In adrenaline and nor adrenaline cells, the vesicles containing granules, are round or oval in shape.

In *C. auratus*, chromaffin cells are innervating by cholinergic nerve fibres. The nerve fibre endings attached to the surface of the chromaffin cells. There are three structures, in the synaptic terminal of innervating chromaffin cells; synaptic vesicles, secretory granules and mitochondria. Synaptic vesicles with electron lucent-contents, 30-60 nm in diameter and secretory granules with dense-cored vesicles, 65-110 nm in diameter, are two types of secretory structure. Synaptic vesicles usually clustered close to synaptic membrane, discharge their contents including acetylcholine (as neurotransmitters) at the synaptic thickening (called active zone) in the presynaptic terminal into the synaptic cleft by exocytosis (Fig. 4-6). Secretory granules usually discharge their contents including neuropeptides in non-synaptic area of nerve ending by exocytosis (Fig. 6). Glia cell usually is present in the peripheral of synaptic terminal (Fig. 8). In chromaffin cells, in addition to peripheral synaptic terminal form, in which secretory granules release their contents in undifferentiated regions of the terminal

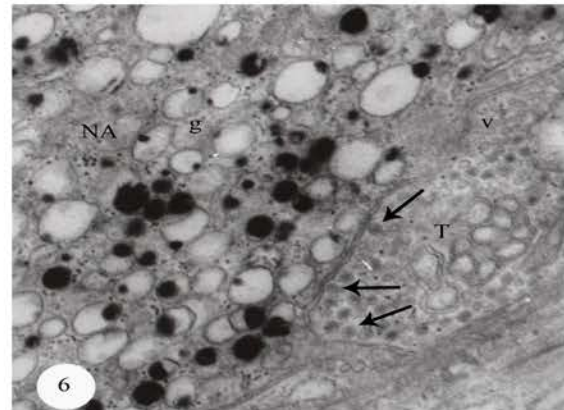


Fig. 6: Electron micrograph of synaptic terminal indicating that secretory granules (arrows) are attached to terminal membrane in every area. NA, nor adrenaline cell; g, granules of nor adrenaline cell; T, synaptic terminal; v, synaptic vesicle

membrane, there is another form of synaptic terminal between several chromaffin cells, which surrounded it (Fig. 7), it seems that in this form of synaptic terminal, secretory granules release their contents on every area of terminal membrane. Occasionally a chromaffin cell is supplied by several nerve endings, also nerve terminal may be connect with more than one chromaffin cells. There are some mitochondria in different shapes in the synaptic terminal (Fig. 5), they play a role in nerve ending functions.

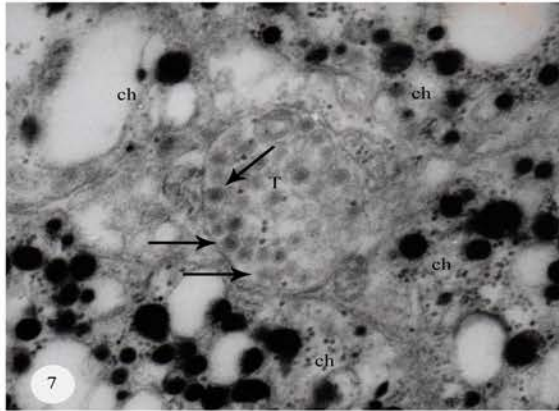


Fig. 7: Indicating synaptic terminal (T) surrounded by several chromaffin cells (ch), that innervated these cells. Secretory granules (arrows) in the synaptic terminal, may be discharge their contents into space between terminal membrane and chromaffin cells in every area of cells

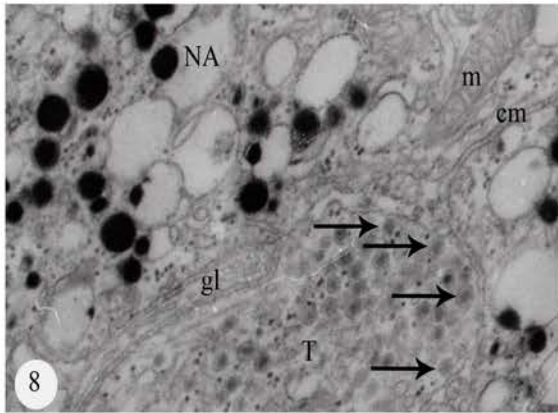


Fig. 8: Electron micrograph of synaptic terminal (T), indicating that secretory granules (arrows) discharge their contents in non specialized area of the synaptic terminal (T). NA, nor adrenaline cell; m, mitochondria; gl, glia cell surrounded synaptic terminal; cm, cell membrane

### DISCUSSION

In fish the homologue of mammalian adrenal cortex is called interrenal tissue and that of the medulla is called chromaffin tissue. The anatomy and situation of adrenal gland of fish have been studied by researchers including Chester-Jones and Bellamy (1964) and Withes (1992). Synaptic terminals ultrasturally have been investigated by Gallo *et al.* (1993), Scheuermann (1993) and Imagawa *et al.* (1996).

Results obtained from the present investigation indicated that in *C. auratus*, chromaffin tissue consists of two kinds of different of secretory cells, adrenaline and nor adrenaline cells. The chromaffin cells are generally more uniform in appearance and are rounded oval in shape. They are organized into cords or small clumps and surrounded by nerves, connective tissue and blood vessels. The occurrence of chromaffin cells close to the blood vessels indicating relationship between these two structures and also it is an evidence shows that the chromaffin cells release their contents, catecholamine hormones into blood circulation. Both types of cells containing vesicles oval or rounded in shape. The vesicles of two types of different cells possess granules in different size and diameters. According to Cormack (1987), the granules of one kind of cells contain adrenaline and those of the other kind, nor adrenaline. Catecholamin hormones adrenaline and nor adrenaline, from chromaffin cells, are released into circulation, during different stressful positions (Hathway *et al.*, 1989). The presence of numerous mitochondria in different shapes, with tubulovesicular cristae in the cytoplasm of chromaffin cells, probably produces the energy for activities of the cells during synthesis of catecholamine hormones. The existence of vesicles in different sizes and densities close to the Golgi complex suggested that the origin of chromaffin granules may be Golgi complex with co-operation of rough endoplasmic reticulum. The granules first condense in tiny microvesicles near tightly packed rough endoplasmic reticulum. These appear to coalesce increasing in electron density with increase in size. Rocha *et al.* (2001), reported that in *Brycon cephalus*, other one of as adrenaline cells, contains vesicles with electron dense granules and the other one as adrenaline cells contains small vesicles, with electron-lucent granules. The frequency of synaptic connection on the carp chromaffin, has been found by Imagawa *et al.* (1996). They reported the frequency of nerve ending differed between each portion of carp head kidney.

In *C. auratus*, the chromaffin cells are innervated by cholinergic nerve fibres. Acetylcholine as a neurotransmitter secreted by the nerve ending synapsing on chromaffin cells, whose induces stimulation of the cells. There are electron-lucent synaptic vesicles and secretory granules within the presynaptic terminal. The synaptic vesicles are clustered in vicinity of the presynaptic membrane in the synaptic terminal and are thought to discharge their contents into the synaptic cleft by exocytosis. Secretory granules which are often larger than from synaptic vesicles have been known as storage site of peptides (neuropeptide) (Schultzberg *et al.*, 1987), neuropeptides, have also been known as neurotransmitters.

In neurons forming conventional synaptic contacts, exocytosis of synaptic vesicles thought to take place within specialized region, associated with presynaptic thickening (so called active zone) (Heuster, 1989). In contrast, exocytosis of secretory granules, more occurs in unspecialized areas of the terminal membrane. During exocytosis, vesicles membrane are fused with surface of terminal membrane in the present of  $Ca^{+2}$  and vesicles release their contents as acetylcholine into synaptic cleft (Guyton, 1991), diffuse to the postsynaptic membrane bind to receptors on the postsynaptic membrane and change the permeability of membrane, transmitting information between the different cells. When vesicles diffused to terminal membrane their surfaces tear by cell membrane and induced exocytosis. As indicated in Fig. 7, in *C. auratus* chromaffin cells innervated by more than one synaptic terminal, this position has been found in mammals by Tomlinson and Coupland (1990), but the nerve ending was not found in all chromaffin cells in *C. auratus*. The existence of mitochondria within the synaptic terminal, suggested that, they probably produce energy for synthesis of neurotransmitters in the nerve ending (Guyton, 1991). In conclusion, in *C. auratus*, as indicated in Fig. 7, in which a synaptic terminal surrounded by 3 chromaffin cells, secretory granules discharge their contents in each area of terminal membrane in unspecialized region, whereas, exocytosis of synaptic vesicles occur in specialized area in the presynaptic thickening.

## REFERENCES

- Chester Jones, I. and D. Bellamy, 1964. Hormonal mechanism in the regulation of the vertebrate body with special reference to the adrenal cortex. Symp. Soc. Exph. Biol., 18: 195-236.
- Cormack, D.H., 1987. The Endocrine System (in Ham's Histology). JB. Lippincott Company, Philadelphia, pp: 591-620.
- Epple, A. and I.C. Potter, 1985. The Cardiovascular chromaffin cell system of Southern Hemisphere Lamprey, *Geotria australis*. J. Morphol., 183 (2): 225-231.
- Gallo, V.P., A. Civinini, L. Mastrolia, G. Leitner and S. Porta, 1993. Cytological and biochemical studies on chromaffin cells in the head kidney of *Gasterosteus aculeatus* (Teleostei: Gasterosteidae). Gen. Comp. Endocrinol., 92 (2): 133-142.
- Guyton, A.C., 1991. The Automatic Nervous System. Textbook of Medical Physiology, W.B. Saunder Company, pp: 667-678.
- Hathway, C.B., J.E. Brinn and A. Epple, 1989. Catecholamine release by catecholamines in the eel does not require the presence of brain or anterior spinal cord. J. Exp. Zool., 249 (3): 238-242.
- Heuster, J.E., 1989. Review of electron microscopic evidence favouring vesicle exocytosis as the structure basis for quantal release during synaptic transmission. Q. J. Exp. Physiol., 74 (6): 1051-1069.
- Imagawa, T., H. Kitagawa and M. Uehara, 1996. The innervation of the chromaffin cells in the head kidney of the carp, *Cyprinus carpio*; regional differences of the connections between nerve endings and chromaffin cells. J. Anat., 188 (1): 149-156.
- Lamouche, S. and N. Yamaguchi, 2001. Role of PAC 1 receptor in adrenal catecholamine secretion induced by PACAP and VIP *in vivo*. Am. J. Physiol., 280 (2): R510-R518.
- Montpetit, C.J. and S.F. Perry, 2000. Vasoactive intestinal polypeptide and pituitary adenylate cyclase activating polypeptide-mediated control of catecholamine release from chromaffin tissue in *Oncorhynchus mykiss*. J. Endocrinol. Metabol., 166 (3): 705-714.
- Perry, S.F. and N.J. Bernier, 1999. The acute humoral adrenergic stress response in fish facts and fiction. Aquaculture, 177 (1-4): 285-295.
- Rocha, R.M., H.S. Leme-Dos Santos, C.A. Vicentini and C. Da Cruz, 2001. Structural and ultrastructural characteristics of interrenal gland and chromaffin cells of Matrinxa, *Brycon cephalus* Gunther 1869 (Teleostei: Characidae). Anat. Histol. Embryol., 30 (6): 351-355.
- Scheuermann, D.W., 1993. Comparative morphology, cytochemistry and innervation of chromaffin tissue in vertebrates. J. Anat., 183 (2): 327-342.
- Schultzberg, M., J.M. Landsberg, T. Hokfelt, L. Terenius, J. Brandt, R.P. Elde and M. Goldstein, 1987. Enkephaline-like immunoreactivity in gland cells and nerve terminals of the adrenal medulla. Neuroscience, 3 (12): 1169-1186.
- Tomlinson, A. and R.E. Coupland, 1990. The innervation of the adrenal gland. IV. Innervation of the rat adrenal medulla from birth to old age. A descriptive and quantitative morphometric and biochemical study of the innervation of chromaffin cells and adrenal medullary neurons in Wistar rats. J. Anat., 169: 209-236.
- Withes, P.C., 1992. Endocrinology (adrenal glands). Comparative Animal Physiology, pp: 548-552.
- Young, J.Z., 1993. The autonomic nervous system of Selachians. Q. J. Microscop. Sci., 75: 571-624.
- Youson, J.H., 1976. Fine structure of granulated cells in the posterior cardinal and renal veins of *Amia calva* L. Can. J. Zool., 54 (6): 843-851.